EQUIPMENT USED FOR THE CULTURE OF LARVAL PENAEID SHRIMP AT THE NATIONAL MARINE FIHERIES SERVICE GALVESTON LABORATORY 1/

B. R. Salser and C. R. Mock National Marine Fisheries Service Gulf Coastal Fisheries Center Galveston Laboratory, Galveston, Texas 77550

Contribution No. 397, National Marine Fisheries Service, Gulf Coastal Fisheries Center, Galveston Laboratory, 4700 Avenue U, Galveston, Texas 77550.

#### ABSTRACT

A culture system used at the Galveston Laboratory for rearing larval penaeid shrimp features: (1) a conical-bottomed rearing tank, (2) air-lift pumps, and (3) a recirculating filter. Recent changes in the techniques have been made which permit the concentrating, freezing and storing of specific diatoms used as food for the protozoeal stages. The system includes equipment for hatching Artemia in sufficient numbers to feed the mysis stages. Some of the advantages of this system are (1) reliable high density culture, (2) efficient utilization of food, and (3) low operation costs.

#### INTRODUCTION

For the past 8 years research on techniques for the culture of larval penaeid shrimp has been conducted at the National Marine Fisheries Service Galveston Laboratory. Research has been directed toward definition of optimum temperatures, salinities, foods, and population densities as well as refinement of rearing facilities and procedures. Utilizing the results from this research dependable hatchery procedures have been developed.

Mock and Murphy (1970) described the basic hatchery techniques now in use at the Galveston Laboratory. With modifications described in this paper, highly reliable production of postlarval shrimp has been achieved. Population densities as high as 260 shrimp per liter have been reared from egg to the postlarval stage with survival ranging from 65% to 85%.

The purpose of this paper is to describe equipment used at the present time.

#### HATCHERY EQUIPMENT

Since the basic physical design of the hatchery has been described (Cook and Murphy, 1969; Mock and Murphy, 1970), several modifications have been made. These changes include the design of the rearing tanks, modification of the aeration and filtration equipment, and the addition of equipment for concentrating and storing algae.

The present hatchery is equipped with conical-bottomed rearing tanks, air-lift pumps, and recirculating filters (Mock, personal communication). The rearing tank is fiberglass and has a total capacity of 1,900 liters (Figure 1). This tank has an overall depth of 165 cm and a diameter of 137 cm. The bottom cone is 43 cm deep and is inclined at a 32° angle. Four 6.4-cm x 6.4-cm angle iron beams support the tank 56 cm above the floor making the total height 221 cm. The bottom cone tapers to a flat 10-cm surface in which a bulkhead fitting is installed.

Necessary plumbing attached to the tank includes a 5.1-cm chemical sink plastic bulkhead fitting secured to the fitting platform. A 5.1-cm plastic gate valve is attached to this fitting with a 5.1-cm polyvinyl chloride (PVC) close nipple and a 5.1-cm PVC 90° elbow. A 5.1-cm hose adapter is used for draining.

The interior of the tank is fitted with four modified airlift pumps and six free air-stones. The use of these air-lift pumps and air-stones results in aeration, water circulation, and uniform distribution of larvae, food, oxygenated water, and wastes. The air-lift pump, a modification similar to that described by Spotte (1970), is constructed of 3.8-cm service weight sewer pipe and fittings. A 66-cm length of pipe with a 0.6-cm slot cut linearly along the bottom is placed along the slope of the bottom cone. This pipe is attached by a 45° elbow and coupling to a 99-cm pipe extending vertically to the water surface. At the surface of the water a 90° elbow is attached to give a directional flow pattern. A size 10 rubber stopper cut diagonally 0.6-cm from the lower edge is inserted into the open end of the slotted pipe. To complete the air-lift pump a 0.6-cm air line is extended through a 0.6-cm hole in the 90° elbow and down the center of the pipe to an air-stone at the lower end of the slotted pipe. The four air-lift pumps are attached to the rearing tank equidistant along its circumference with 3.8-cm plastic pipe clips and 0.6-cm nylon nuts and bolts. For more versatility, a short vertical pipe is used and a 3.8-cm 90° elbow or tee on this pipe creates the circulation when the tank is half full.

Filtration methods used in the system have also been developed to provide for more dependable and efficient culture. The filter system consists of a center screen or standpipe, a closed 5-ym cold water filter, and an air-lift pump. The center screen illustrated in Figure 1 is used when it is necessary to exchange, drain, or recirculate water through the 5-µm filter. The screen material is either nylon or polyethylene cloth with a mesh size of 0.16 mm. This material is formed into a sleeve 25 cm in diameter and 71 cm long. At the lower end, the sleeve is attached to a 25-cm cone of screening material which tapers to a 10-cm diameter at the bottom. Epoxy painted plywood disks 25.0 cm by 1.3 cm and 10.0 cm by 1.3 cm are used as support for screen at the top and bottom respectively. These disks are glued to a 5.1-cm PVC pipe. The lower end of this pipe adapts to a 5.1-cm chemical sink type, plastic standpipe to seal with the bulkhead fitting. The upper end of this pipe extends above the water surface when in place. Additional support for the screen consists of three rigid plastic collars, 3.8 cm wide by 102 cm long, spaced at 30-cm intervals along the sleeve between the disks. The center pipe is perforated with 1.3-cm holes at 2.5-cm intervals between the wooden disks.

At times when water drainage or filtration is not necessary, a standpipe is used in place of the filter screen. This standpipe is a 2.5-cm PVC and is adapted to a 5.1-cm chemical sink type, plastic standpipe 25 cm long. This standpipe also extends above the water surface and is held in place with rubber bands. When the standpipe is in place, wastes cannot accumulate in the dead space between the bulkhead fitting and the valve.

For filtration purposes while larvae are in the naupliar and mysis stages a commercial 5-µm in-line cold water filter cartridge is used. The cartridge is converted from a 1.9-cm to a 2.5-cm inlet and outlet by use of 1.9-cm to 2.5-cm hose adapters for convenient connection to the gate valve and the air-lift pump. Flexible 2.5-cm plastic tubing is used to connect the filter cartridge to the 2.5-cm hose adapter at the gate valve.

The air-lift pump that lifts the filtered water to the surface of the tank is constructed of 2.5-cm plastic tubing and hose fittings. A 173-cm length of the tubing is connected directly to the hose adapter at the filter cartridge outlet. At the upper end of this length of vertical pipe, a 2.5-cm hose connector elbow is used to direct the flow horizontally above the tank through 8 cm of plastic tubing. A second elbow then is used to direct the flow down and at an angle to blend with the tank's circulation pattern. A 0.6-cm air line enters the vertical plastic pipe through a 0.6-cm hole in the first elbow and is connected to an air-stone at the lower end of the plastic pipe.

ALGAL FOOD CONCENTRATION, FREEZING, AND FEEDING EQUIPMENT

Griffith, Kenslow and Ross (1973) described techniques used to provide reliable, high quality, mass culture of algal foods. To improve efficiency in the feeding process, a method for concentrating and preserving these foods was developed (Mock, 1971) and an alternate method for freeze-drying was described (Brown, 1972).

Today all algal foods are concentrated and frozen prior to the actual larval rearing experiment. The equipment used is a dairy cream separator and a standard home freezer. A De Laval Model  $108^{2/}$  cream separator with a bowl diameter of 17.5 cm and standard working speed of 7,500 revolutions per minute is used to concentrate the algae. The aglae accumulates along the outer wall of the centrifuge bowl similar to sludge during the milk separation process. With this separator, flow rates of 3 to 4 liters per minute are used for optimum efficiency of time and reclaim. For example Skeletonema sp., a chain forming diatom having a diameter of 2-4 um, can be concentrated at this flow rate to a density of about 500-600 million cells per milliliter with a recovery of 80% or more of the cells in a culture. Tetraselmis chui Butcher, a

 $<sup>\</sup>frac{2}{}$  The use of trade names in this publication does not imply endorsement of commercial products.

single-celled flagellate with a length of 12-15 µm can be concentrated to about 40-60 million cells per milliliter with recovery of 70% or more. Higher speeds and/or lower flow rates can result in significantly lower reclaim efficiency. After use the separator is dismantled and the algae is removed from the bowl.

Because this concentrate is greatly reduced in volume, storage is easily accomplished in a standard home freezing unit. The freezer now in use maintains a temperature of approximately -11°C; however, other units have been used successfully at temperatures ranging from -4° to -20°C. The concentrate is frozen in polyethylene containers at known cell counts and predetermined volumes for feeding in the rearing tanks. Concentrates stored 2 years have been used, resulting in larval survival rates as high as 93% from the 5th naupliar stage to the first mysis stage.

As mentioned by Mock (1971), continuous feeding equipment is being used at the Galveston Laboratory. A diagram of a typical continuous feeding apparatus utilizing a metering pump is illustrated in Figure 1. Any non-metalic chemical metering pump that delivers flow rates of 5-10 milliliters per minute is acceptable. Depending on accuracy desired, pumps meeting these specifications are available for \$20 to \$300.

The algal reservoir is a glass beaker with a capacity of 20 liters. To maintain a uniform concentration of algae in the reservoir a magnetic stirrer is used. Finally, the storage reservoir is placed in a refrigerator to maintain the algae at about 4°C.

#### ARTEMIA HARVESTING EQUIPMENT

Research has suggested the need for animal protein in the diet of the mysis stage of penaeid shrimp (Wickins, 1972). Many organisms have been tested, and several have proven to be suitable foods for the mysis stage. Few, however, are well suited for high density, reliable mass culture. Freshly hatched <u>Artemia</u> sp. is the only economical source of animal protein found to be suitable for use in our system.

Two authors (Sorgeloos and Persoone, 1973; Nash, 1973) have described sophisticated techniques and equipment for reliable, high density hatching of Artemia sp. However, at the Galveston Laboratory we use a relatively simple method to hatch large quantities of Artemia on a daily basis. The equipment necessary for our technique is illustrated in Figure 2. The hatching tank is identical to the larval shrimp rearing tank except that it is aerated only with suspended air-stones along the wall and at the center. A 2.5-cm bulkhead fitting at the bottom provides drainage through a 2.5-cm valve. A recent modification to this procedure is the use of intensified lighting at 2,000 lux to increase hatching (Sorgeloos and Persoone, in press).

Concentration of the Artemia nauplii is a valuable technique in feeding the shrimp and storing Artemia; simple harvesting equipment is used in this concentration. A screen bag 16 cm in diameter and 46 cm deep is constructed of 0.16-mm mesh nylon or polyethylene material. The harvesting screen is supported by a column of Vexar plastic netting. The screen plus support are placed inside a 10-liter plastic bucket. As the Artemia and water flow from the valve through the screen, the larvae are retained. When the bucket overflows Artemia larvae are concentrated in the water remaining in the bucket. Artemia can be stored up to 72 hr at about 4°C with proper aeration at densities of 5,000-6,000/ml.

#### ADVANTAGES OF THE GALVESTON CULTURE SYSTEM

The primary advantage of the larval culture techniques employed at the Galveston Laboratory is the reliability of the system. Better control of environmental conditions has made this possible. Due to high population densities and the rearing tank design, food utilization is efficient. Finally, the operation costs are low because of mechanization.

Strict environmental control has been necessary to rear larvae reliably at high densities (to 260 shrimp per liter). The new rearing tank has fewer areas in which particulate wastes accumulate. Circulation of the water is accomplished with air-lift pumps providing both better

aeration and better distribution of larvae and food. With the new filter design control of water quality is accomplished through exchange, or recirculation. Due to the relatively low water volumes necessary for high density culture, it is possible to maintain an optimum temperature of  $28.5^{\circ} \pm 0.5^{\circ}$ C, and salinity is easily adjusted to optimum levels of 28 to 30o/oo with synthetic sea salts or fresh water. Furthermore, the limited water requirements and the completely enclosed system afford easy restriction of undesirable organisms.

Efficient utilization of food is possible because the air-lift pumps continuously lift algal food from the bottom to the surface of the water for additional exposure to the shrimp feeding in the water column. The feeding device also contributes to feeding efficiency by metering algae into the tank in small volumes continuously.

A final advantage of the techniques used at the Galveston Laboratory is the low operational costs. The tank design, along with air-lift pumps, and the filtration equipment, offer a nearly maintenance-free system requiring little labor. High rearing capacities in low water volumes reduce requirements for labor, pumps, heating equipment and food. By concentrating and freezing specific diatoms, both advance food preparation and continuous feeding are possible, thus reducing labor requirements.

#### SUMMARY

Extensive research has been conducted at the Galveston Laboratory to develop a system to produce large numbers of postlarval shrimp consistently. Previous papers have described the basic equipment and techniques. Recent modifications, however, have resulted in improved survival and a reduction in labor requirements. The new larval rearing tank with the conical bottom is a major modification. Also described are the air-lift pump system, a new filter screen, and the filter system. Furthermore the concentrating, freezing, and continuous feeding of algal foods are recent advances in the techniques used at Galveston. The Artemia sp. hatching system also provides advantages over previously described techniques.

## Key to Figure 1

No.	Description	Measurements
1.	Rearing tank, conical bottom	137 cm diameter x 165 cm depth (cone 43 cm deep, 320 angle)
2.	Angle iron tank supports	6.4 x 6.4 cm x 206 cm
3.	PVC pipe, perforated	200 cm x 5.1 cm (holes 1.3 cm diameter)
4.	Plywood disk, epoxy coated	25 cm diameter x 1.3 cm wide
5.	Plastic collar	102 cm long x 3.8 cm wide
6.	Screen, nylon	0.16-mm mesh
7.	Plywood disk, epoxy coated	10 cm diameter x 1.3 cm wide
8.	Bulkhead fitting	5.1 cm
9.	PVC elbow	5.1 cm
10.	PVC nipple	5.1 cm
11.	Plastic gate valve	
12.	PVC reducer	5.1 cm to 2.5 cm
13.	In-line filter assembly	
14.	MillionAire air-stone	15 cm x 1.9 cm

No.	Description	Measurements
15.	Airline	0.64 cm outside diameter
16.	Vertical Plastic Pipe	99 cm x 3.8 cm
17.	Plastic elbow	90°x 3.8 cm
18.	Plastic elbow	45° x 3.8 cm
19.	Plastic coupling	3.8 cm
20.	Slotted plastic pipe	66 cm x 3.8 cm (slot 64 cm long x 0.64 cm wide)
21.	Air-stone	2.5 cm
22.	Rubber stopper, diagonal cut	Size 10 (cut 0.64 cm from lower edge)
23.	Continuous feed device	
24.	Refrigerator	
25.	Algal storage reservoir	
26.	Magnetic stirrer	

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# Key to Figure 2

No.	Description	Measurement
1.	Hatching tank	137 cm diameter x 165 cm deep (cone 43 cm deep, 32° angle)
2.	Airline	0.64 cm outside diameter
3.	MillionAire air-stone	15 cm x 1.9 cm
4.	Bulkhead fitting and valve assembly	
5.	Drain hose	2.5 cm diameter
6.	Harvesting screen	16 cm diameter x 46 cm deep (0.16 mm mesh)
7.	Screen support	16 cm diameter x 41 cm deep (0.64 mm mesh)
8.	Plastic bucket	10 liter
9.	Harvesting light	60 watt
10.	Fluorescent hatching lights	2 cool white 86-watt, 183-cm tubes

#### LITERATURE CITED

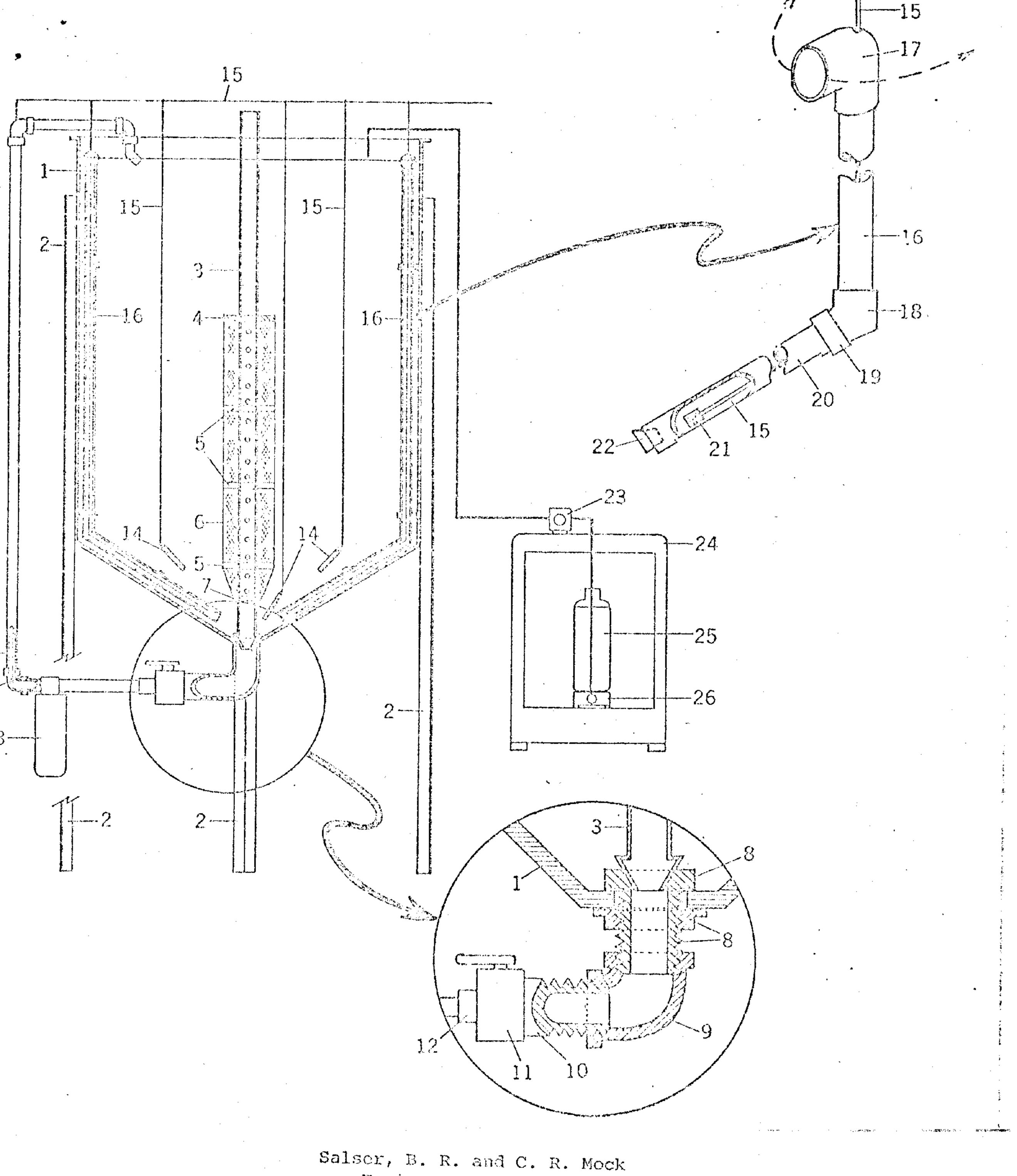
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- Figure 2. Artemia hatching tank and harvesting equipment.



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Equipment used for the culture of larval
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Figure 1. Shrimp larval rearing tank and supporting equipment.

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Figure 2. Artemia hatching tank and harvesting equipment.